Tailored Assays for the Detection of Agroterrorism Viral Agents

P. McCready, S. Messenger, K. Smith, E. Skowrowski, T. McKenna, R. Heckert, A. Ardans, S. Hietala

This article was submitted to CBNP Summer Meeting, Arlington, VA, June 3-5, 2003

May 28, 2003

U.S. Department of Energy



DISCLAIMER

This document was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor the University of California nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or the University of California, and shall not be used for advertising or product endorsement purposes.

This is a preprint of a paper intended for publication in a journal or proceedings. Since changes may be made before publication, this preprint is made available with the understanding that it will not be cited or reproduced without the permission of the author.

This work was performed under the auspices of the United States Department of Energy by the University of California, Lawrence Livermore National Laboratory under contract No. W-7405-Eng-48.

This report has been reproduced directly from the best available copy.

Available electronically at http://www.doc.gov/bridge

Available for a processing fee to U.S. Department of Energy
And its contractors in paper from
U.S. Department of Energy
Office of Scientific and Technical Information
P.O. Box 62
Oak Ridge, TN 37831-0062

Telephone: (865) 576-8401 Facsimile: (865) 576-5728 E-mail: reports@adonis.osti.gov

Available for the sale to the public from U.S. Department of Commerce National Technical Information Service 5285 Port Royal Road Springfield, VA 22161 Telephone: (800) 553-6847 Facsimile: (703) 605-6900

E-mail: <u>orders@ntis.fedworld.gov</u>
Online ordering: <u>http://www.ntis.gov/ordering.htm</u>

OR

Lawrence Livermore National Laboratory
Technical Information Department's Digital Library
http://www.llnl.gov/tid/Library.html



Tailored Assays for the Detection of Agroterrorism Viral Agents

A collaborative effort between:

Paula McCready, Sharon Messenger, Kimothy Smith, Evan Skowrowski Lawrence Livermore National Laboratory

Tom McKenna, Plum Island Animal Disease Center, USDA Robert Heckert, Agricultural Research Service, USDA

Alex Ardans, Sharon Hietala California Animal Health and Food Safety Laboratory





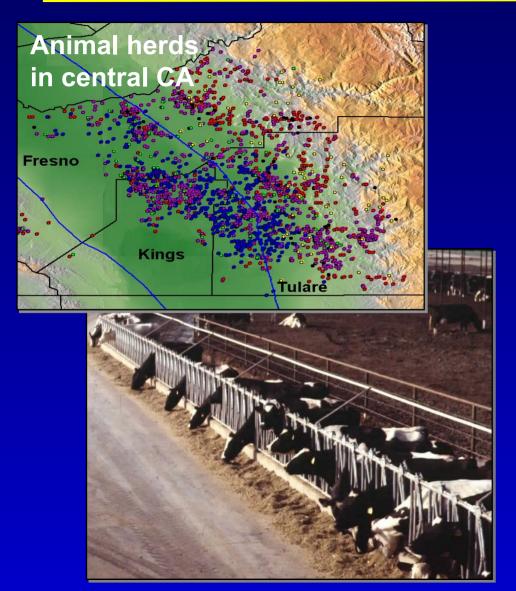
Agroterrorism

...a less publicized, but potentially devastating threat (Henry Parker, NDU)

- ✓ American food & agricultural infrastructure:
 - One-sixth of the national GDP
 - 1 in 8 Americans employed in food production
- **✓** Agricultural infrastructure vulnerable
 - High density production
 - Easily accessible
 - Highly susceptible



New efforts will offer better surveillance and response strategies: rapid rule-outs for FADs



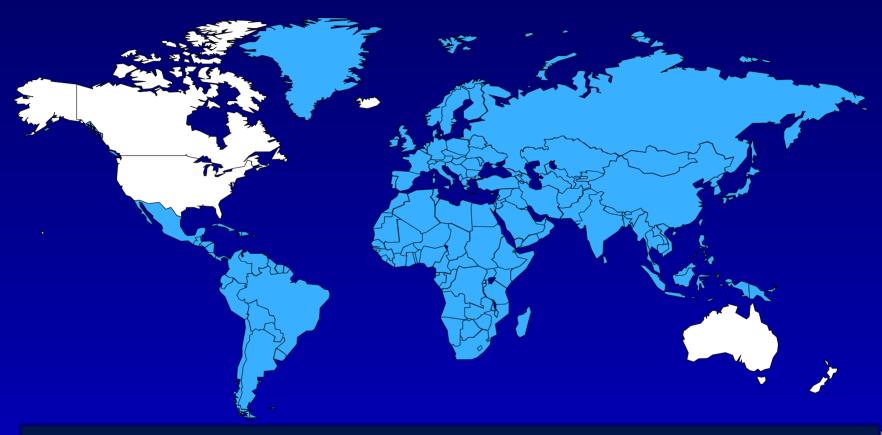
- Animals are highly vulnerable due to dense populations and lack of security
- Rapid diagnostics are needed to discriminate FADs from symptomatic look-alikes that naturally occur in our country
- Nested surveillance?

Motivation

- ✓ Exotic agricultural diseases will be introduced
 - Naturally or intentionally
 - The question is not "if", but "when"
- Early detection is key to minimizing impact
 - Rapid, accurate detection technologies
 - Efficient, secure communication networks



International Disease Status*



Office Internationale des Epizooties List A diseases:
Foot and Mouth, Rinderpest, Classical Swine Fever, African
Swine Fever, BSE, Exotic Newcastle disease, High Path
Avian Influenza

A Coordinated Interagency Effort to Protect Agriculture Rapid detection of exotic animal/plant diseases

- ✓ Program objectives:
 - High degree of specificity
 - Compatible with several detection instruments
 - Validated for multiple plant/animal species
 - Disseminated through USDA/National Animal Health Laboratory Network







Improved Diagnostics

- ✓ Work with collaborators to identify end user needs
 - Diagnostic gaps
 - Priority disease agents
- Leverage current infrastructure to deliver assays quickly
 - Tailored Assay program
 - LLNL whole genome approach
 - High throughput sequencing capability

The new process rapidly evaluates thousands of candidate signatures

Candidate Signatures of Microbes Candidate Computer Screening and signature **Down Selection** evaluation **Wet Chemistry Screening** and Down Selection **Assay Development and Down Selection Assay** evaluation **Assay Validation**

Totals

100,000's computationally screened

~8,500 primer pairs ordered for screening

300 highly specific signatures

80-100 rapid assays

30-60 for validation

Operational Strategy

- ✓ Produce target list
 - 8 viruses/ 2 bacteria
 - FMDV disease "look alikes"
- ✓ Identify diagnostic platforms
 - Specific & rapid
 - Cost effective & capable of high throughput
- Develop signatures for bioassays
 - Sequence target species & near neighbors
 - DNA pipeline for screening and down selection
 - End product is set of unique signatures

Symptoms of foreign animal diseases are often indistinguishable from endemic viruses

Foot and Mouth Disease



Vesicular Stomatitis



Malignant Catarrhal Fever

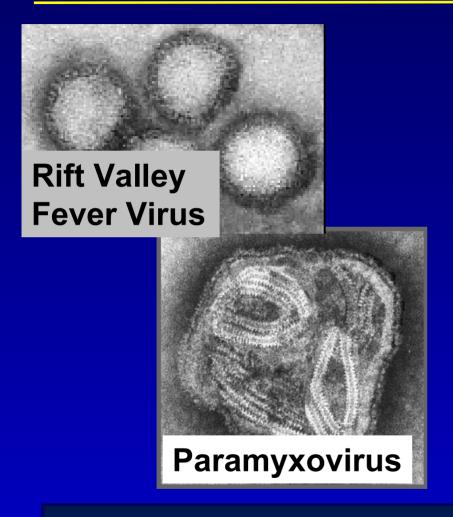
Bluetongue



BVD



This effort has expanded to support USDA on high priority assay development



- Listeria monocytogenes
- <mark>√ Orf</mark>
- ✓ <u>Vesicular Exanthama of Swine</u>
- ✓ Pseudorabies
- ✓ Johne's Disease
- ✓ Rift Valley Fever
- Infectious Bovine Rhinotracheitis
- ✓ Swine Vesicular Disease
- ✓ Malignant Catarrhal Fever
- ✓ Newcastle

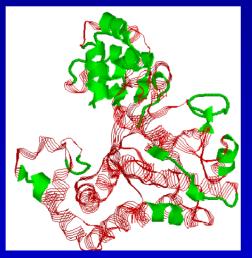
This pathogen assay list was selected by USDA and reflects priorities and coordination with PIADC

Signatures that pass screening are optimized for detection on different real-time PCR platforms



Sequencing efforts will produce the fundamental data upon which diagnostics are based

- Better characterization of pathogen diversity
- Improve throughput
- ✓ Increase cost effectiveness of whole genome sequencing of viruses



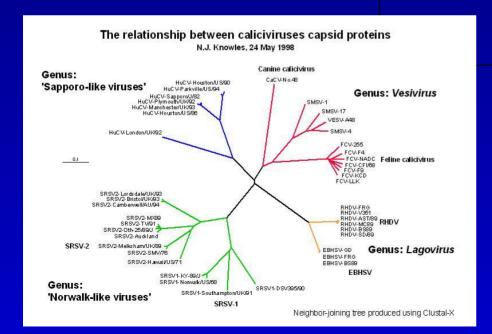
TCACTCCGGC CGACAAAAGC GACAAAGGTT TTGTTCTTGG TCACTCCATA
TCACTCCGGC CGACAAAAGC GACAAAGGTT TTGTTCTTGG TCACTCCATA
TTACTCCAGC TGACAAAAGC GACAAAGGTT TTGTTCTTGG TCACTCCATT
TTACTCCAGC TGACAAAAGC GACAAAGGTT TTGTTCTTGG TCACTCCATT
TCACTCCGGC CGACAAAAGC GACAAAGGTT TTGTTCTTGG TCACTCCATA
TTACTCCAGC TGACAAAAGC GACAAAGGTT TTGTTCTTGG TCACTCCATA

>fmdv.polymerase_P56:XXXNGTVGP XVXXAXXLMXXXXYXXXXXXTFLKDXX RPXXXVRAGKTRXVXVXPVXXILYTXX XXXVWXVDXSAFXXXXXXXXAMNIMXX XVXRTXXGXHPXAEWXLXTLVXTXHA XXNXRXXVXGGMPSXXSATXIXNTILX NXYVLYAXRXHXEGVELDTYTMISXXX DXVVAXXXXXXXXEALKPHXXSLGXTX TPADKSDKGFVLGXSXTXVTFLKXHF XXXXGTGFXKPVMASKTLEAILSFARR GTXQEKLXSVAGLAVHSGPXXYRRLF EPXXGLXEXPSYRSLYLRWVNAVCGD

Justification for sequencing efforts...Improved bioassay signatures

×

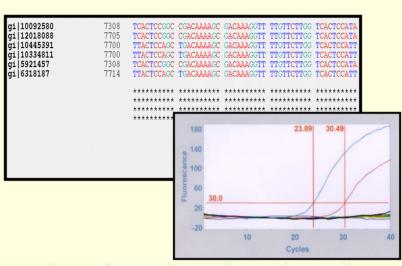
- **✓** Greater accuracy
- Improved stability



✓ Rational approach to select near neighbors for sequencing

LLNL / CAHFS have been collaborating on FMDV / BVD assay development for almost 2 years







	- 1				42 ×		g)	00	VI IV IV V					90 50	9.		
Octo 20	0.00000000	November 2001	December 2001	January 2002	February 2002	March 2002	April 2002	May 2002	June 2002	July 2002	August 2002	September 2002	October 2002	November 2002	December 2002	Janu 20	uary 103

In Silico Development LLNL/CAHFS Initial Assay Testing FADDL-Plum Island

Plum Island-Animal Experiments

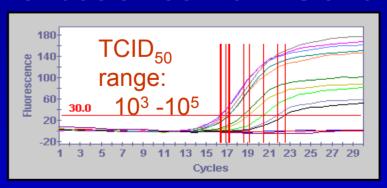
APHIS Validation Pending

FMDV and BVD assays were developed in weeks



Which is FMDV?

- ✓ Rapid assays to rule out symptomatic "look-alike" diseases from FMDV
- Highly sensitive & specific
- Multiplexing has begun
- FMDV assays are in final validation at Plum Island

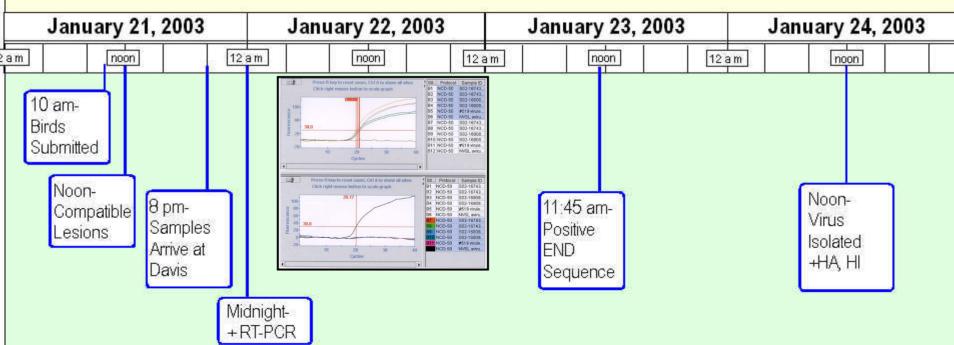


USDA making final decision on how to field assays of this type

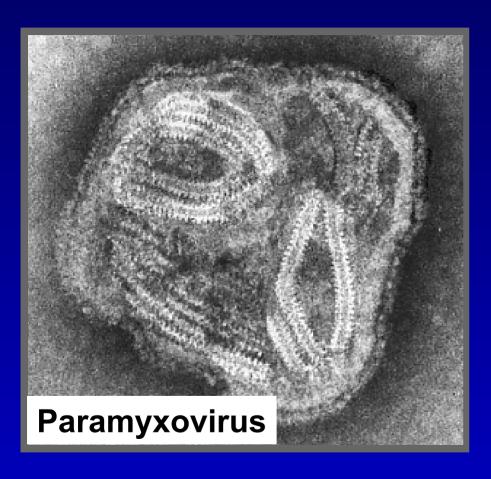
LLNL / CAHFS provide in-depth diagnostic information within 2 days of receiving END suspect field samples







LLNL has demonstrated a capability to rapidly respond to emerging threats

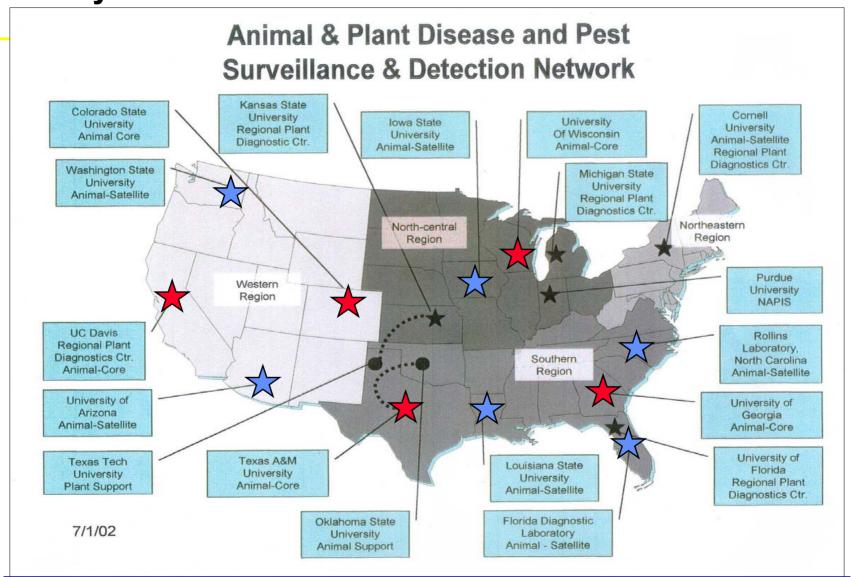


- Oct 13: UCD CAHFS Vet Lab called LLNL regarding Newcastle outbreak in game chickens
- Oct 15: Primers and probes ordered for screening
- ✓ Oct 18: Reagents split between vet diagnostic lab and LLNL screening lab
 - Vet lab will compare new assays with culture assays
 - LLNL will screen in parallel

Current testing takes about a week for feedback.

New process takes 1-2 hours.

USDA is networking animal and plant centers around the country



We are engaging the network for testing and validation

Future Directions

- FY'03 and beyond
 - Increase sequencing capabilities
 - Additional high priority pathogens
 - Denser sampling of near neighbors
 - Continued coordination with end users to field bioassays
 - validation
 - deployment
 - Investigate new technologies
 - High throughput
 - Field capable
 - Alternative DNA assays
 - Orthogonal testing Protein signatures

Acknowledgments



Beth George, Pat Fitch, Paula McCready, Sharon Messenger, Kimothy Smith, Evan Skorowski





Sofi Ibrahim, Peter Jahrling



Robert Heckert, Thomas Mckenna



C.J. Peters, Robert Tesh, Robert Shoppe



Alex Ardans, Sharon Hietala, Mark Thurmund